2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO CARBON TETRACHLORIDE IN THE UNITED STATES

Carbon tetrachloride is a solvent that has been used in the past as a cleaning fluid or degreasing agent, as a grain fumigant, and industrially in the synthesis of refrigeration fluid and propellants for aerosol cans. Although most of these uses have been discontinued, the possibility still exists for carbon tetrachloride to be released to the environment, primarily through industrial processes or old bottles of cleaning agents containing carbon tetrachloride that may still be in the home. Degradation of carbon tetrachloride occurs slowly in the environment, which contributes to the accumulation of the chemical in the atmosphere as well as the groundwater. Carbon tetrachloride is widely dispersed and persistent in the environment, but is not detected frequently in foods.

The general population is not likely to be exposed to large amounts of carbon tetrachloride. Populations living within or very near waste sites, or areas of heavy carbon tetrachloride use would have an increased risk of exposure from contaminated media (air, water, or soil). Those likely to receive the highest levels of exposure are those who are involved in the production, formulation, handling, and application of carbon tetrachloride. Inhalation appears to be the major route of exposure for workers and also for the general population, which may be exposed to carbon tetrachloride in ambient air and from volatilization of contaminated water during showering or bathing. Ingestion via contaminated drinking water is an important route of exposure for the general population not living in areas where carbon tetrachloride is extensively used. Dermal contact, principally from showering or bathing, has not been shown to be a significant route of exposure to carbon tetrachloride.

Most carbon tetrachloride released to the environment is expected to volatilize rapidly due to its high vapor pressure. Outdoor measurements in several areas of the United States have reported average concentrations of carbon tetrachloride in air between 0.6 and 1.0 $\mu g/m^3$. Typical indoor concentrations in homes in several U.S. cities were about 1 $\mu g/m^3$ (0.16 ppb), with some values up to 9 $\mu g/m^3$ (1.4 ppb). Indoor concentrations in indoor air were thought to be higher than in outdoor air because of the presence of carbon tetrachloride in building materials or household products. The majority of domestic water supplies contain carbon tetrachloride at concentrations below 0.5 µg/L. Children are expected to be exposed to carbon tetrachloride by the same routes that affect adults. Since carbon tetrachloride has a low affinity for adsorption onto soil and dust particles, the risk of exposure for small children from ingesting

soil or dust is likely to be low. The average daily intake of carbon tetrachloride for the general population is estimated as 0.1 μg/kg/day from inhalation exposure and 0.01 μg/kg/day from ingesting drinking water containing typical low concentrations of the chemical.

See Chapter 6 for more detailed information regarding concentrations of carbon tetrachloride in environmental media.

2.2 SUMMARY OF HEALTH EFFECTS

As a volatile halogenated alkane, carbon tetrachloride has depressant effects on the central nervous system that are most significant at high exposure levels. Carbon tetrachloride also produces irritant effects on the gastrointestinal tract. Most other toxic effects of absorbed carbon tetrachloride are related to its metabolism by mixed function cytochrome P-450 oxygenases (in humans, primarily CYP2E1, but also CYP3A). The liver is the most sensitive target in exposed humans and animals, independent of the route of administration, because of the abundance of CYP2E1 and other cytochromes. The kidneys are also sensitive targets in humans and animals. Carbon tetrachloride has been shown to be carcinogenic in animals following chronic inhalation or oral exposure.

Studies in animals, combined with limited observations in humans, indicate that the principal adverse health effects associated with inhalation exposure to carbon tetrachloride are central nervous system depression, liver damage, and kidney damage. Case reports in humans and studies in animals indicate that the liver, kidney, and central nervous system are also the primary targets of toxicity following oral exposure to carbon tetrachloride. Gastrointestinal irritation has been frequently noted following accidental ingestion in humans. Limited dermal data suggest that carbon tetrachloride absorbed through the skin can cause, in addition to skin irritation, gastrointestinal effects such as nausea and vomiting and neurological effects such as polyneuritis in humans, and liver damage in animals. Based on the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values identified in the animal studies, the liver appears to be the most sensitive target. Several types of liver effects have been observed in humans and laboratory animals. At lower adverse effect levels, hepatocytes accumulate lipids, resulting in cellular vacuolization and fatty degeneration. At higher exposure levels, hepatocellular necrosis (cell death), fibrosis, and cirrhosis are observed. Hepatic carcinogenicity has been observed in laboratory rodents following chronic-duration inhalation or oral exposure to carbon tetrachloride. In animal studies, kidney effects are typically observed at higher doses than hepatic effects. Tubular cell degeneration and fatty accumulation have been observed in the kidneys in animal studies.

Human case reports indicate that high oral or inhalation exposures sufficient to cause renal failure (progressive uremia and electrolyte retention) may cause delayed secondary damage (edema) to the lungs. Central nervous system effects following inhalation or oral exposure include headache, weakness, lethargy, stupor, blurred vision, and coma. High-level inhalation or oral exposure is associated with mild hematological effects, primarily anemia in humans and animals, and reduced platelet function (clotting efficiency) in animals. One accidental ingestion case resulted in cardiac arrhythmia, which was reversible. Suppression of immune function (reductions in IgM antibody-forming cell activity, T-cell activity, lymphocyte counts, or host resistance to bacteria) has been observed in animals exposed short-term to moderate oral doses.

No studies were located regarding reproductive effects in humans after exposure to carbon tetrachloride and the available human data for developmental effects are limited to epidemiological studies of pregnancy outcomes in women exposed to carbon tetrachloride and other halogenated hydrocarbons in drinking water. These data are inadequate for establishing a causal relationship between carbon tetrachloride exposure and developmental toxicity in humans. In animals exposed by inhalation for intermediate durations, reproductive effects included decreased fertility and testicular atrophy. In one study, no effect on reproduction was detected in rats that ingested carbon tetrachloride at a dose slightly higher than the LOAEL for hepatic effects for several weeks. Developmental studies in animals exposed by inhalation or ingestion revealed that carbon tetrachloride was not teratogenic or ingestion. However, complete litter loss occurred in some rats orally exposed at doses that produced clear maternal toxicity. It is not known whether litter loss is the result of toxicity to the fetus or to the placenta.

The following sections discuss significant effects resulting from exposure to carbon tetrachloride in greater detail: neurological, hepatic, renal, and cancer.

Neurological Effects. Studies in humans revealed that depression of the nervous system usually appears quite rapidly following inhalation or oral exposure to carbon tetrachloride. Central nervous system depressant effects were reported following occupational inhalation exposures at approximately 20–125 ppm and following single oral doses of 114 mg/kg or higher. The most characteristic signs are headache, vertigo, confusion, lethargy, and stupor. High exposures (a single oral dose of 4,800 mg/kg) may also lead to marked depression of respiration and cardiac output and coma. Simple depressant effects appear to be reversible. Central nervous system depression has also been reported in animals exposed to carbon tetrachloride vapors.

In addition to clinical signs of central nervous system depression, neurohistopathological effects (primarily fatty degeneration and necrosis) have been detected in the brain and peripheral nerves in humans following lethal exposure. Exposure of rats, monkeys, or guinea pigs to concentrations of carbon tetrachloride up to 400 ppm for over 10 months had no overt neurobehavioral effect, but caused degenerative changes in sciatic and optic nerves in rats at concentrations as low as 50 ppm.

Hepatic Effects. Hepatotoxicity is the primary effect of exposure to carbon tetrachloride by any route in humans and animals. Liver injury is detectable by clinical signs (jaundice, swollen and tender liver), biochemical alterations (elevated levels of hepatic enzymes in the blood, loss of enzymic activities in the liver), or histological examination (fatty degeneration and necrosis of central hepatocytes, destruction of intracellular organelles, fibrosis, cirrhosis). Elevated levels of serum enzymes may provide evidence of hepatocellular injury in the absence of clinical signs, as was observed in workers occupationally exposed at intermediate-to-chronic durations at levels between 1.1 and 12 ppm. Degeneration or necrosis of the liver was noted in humans following acute inhalation exposure at 250 ppm or acute oral exposure at ≥110 mg/kg. In humans, acute lethal inhalation or oral exposures were associated with massive liver necrosis and steatosis. In rats, centrilobular vacuolization was observed at an acute oral dose of 20 mg/kg/day, whereas necrosis was observed at 80 mg/kg/day. Hepatic necrosis was also observed in guinea pigs following acute dermal exposure at 513 mg/cm^2. In chronic studies, fatty change was observed at 5 ppm in rats, whereas fibrosis and cirrhosis developed at 25 ppm; in the same study, mice did not show fibrotic changes, but rather necrosis. These species differences may be related to the differential involvement of tumor necrosis factor alpha, which may facilitate necrosis, or transforming growth factor beta, which is an initiator of fibrosis.

It is widely agreed that the reason for the special sensitivity of the liver to carbon tetrachloride toxicity is the inherently high rate of metabolism of carbon tetrachloride by this tissue, presumed to be associated with the high abundance of CYP2E1, particularly concentrated in the centrilobular zone. This hypothesis was verified for mice in a study that administered 1,590 mg carbon tetrachloride/kg body weight by intraperitoneal injection to CYP2E1 knockout mice *(cyp2e1$^{-/-}$)*. Livers of knockout mice failed to develop hepatotoxicity that was observed 24 hours after treatment in treated mice expressing CYP2E1: elevated serum enzyme levels (alanine aminotransferase and aspartate aminotransferase) and histopathology (centrilobular parenchymal degeneration and perivenular vacuolation). In humans also, CYP2E1 is the primary enzyme responsible for metabolizing carbon tetrachloride at environmentally relevant concentrations, but others, particularly CYP3A, are also involved at higher concentrations. The reactive metabolites (trichloromethyl free radicals) generated by the oxidation of carbon tetrachloride are

believed to trigger a spectrum of hepatocellular damage. Mechanisms that appear to be involved include direct binding of reactive metabolites to cellular proteins, peroxidation of unsaturated membrane lipids, and alterations in intracellular calcium levels. The outcome of any carbon tetrachloride-induced injury has been demonstrated to depend on several factors, including the induction of P450 enzymes and the presence of antioxidants and interactions with other chemicals.

Renal Effects. Injury to the kidney is also observed in many reports of carbon tetrachloride toxicity in humans, often at the same exposure levels that cause hepatic injury. The principal clinical signs in severe cases are oliguria or anuria, with resultant azotemia and edema, leading in turn to hypertension and pulmonary edema. Cells of the proximal tubule are most clearly injured by carbon tetrachloride, probably because of high content of cytochrome P-450. Renal injury is observed in animal studies, but usually at higher doses with lesser severity than in humans. The reasons for these species differences are not clear, but might be related to differences in carbon tetrachloride metabolism by different organs (liver or kidney).

Cancer. There are a few reports of cancer in people who have been exposed to carbon tetrachloride, but these data alone are not sufficient to show that carbon tetrachloride causes cancer in humans. Suggestive data in humans comes from occupational case-control studies that found positive associations between exposure to carbon tetrachloride and mortality from several types of cancer (lymphosarcoma, lymphatic leukemia, non-Hodgkin's lymphoma, or multiple myeloma). There is convincing evidence that exposure to carbon tetrachloride leads to hepatic tumors in rodents exposed by inhalation or dosed orally. The lowest cancer effect levels were observed for mice: 25 ppm by inhalation and 20 mg/kg/day orally. The incidence of adrenal pheochromocytomas was increased in mice exposed by inhalation.

The carcinogenicity of carbon tetrachloride is related to its metabolism. Although most *in vivo* genotoxicity assays for carbon tetrachloride were negative, lipid peroxidation products were shown to form DNA adducts in the liver of rats exposed orally and in liver, forestomach, lung, colon, or kidney of rats exposed by intraperitoneal injection. DNA damage, evaluated electrophoretically, occurred in the liver, but not in the stomach, kidney, bladder, lung, brain, or bone marrow of mice 24 hours after a single oral dose of 1,000 mg/kg; no DNA damage occurred at 500 mg/kg. DNA damage in the liver was probably secondary to liver necrosis and hepatocellular degeneration. There is some evidence that carbon tetrachloride may also cause cancer by a nongenotoxic mechanism involving cellular regeneration. Mild hepatic necrosis stimulates cell division processes; the resulting increase in cell proliferation could result in either the replication of unrepaired DNA damage or the induction of additional errors during the

replication process, both of which can produce heritable mutations that may result in an initiated preneoplastic cell.

The U.S. Department of Health and Human Services has determined that carbon tetrachloride may reasonably be anticipated to be a carcinogen. IARC has classified carbon tetrachloride in Group 2B, possibly carcinogenic to humans. EPA has determined that carbon tetrachloride is a probable human carcinogen and derived an oral slope factor of $1.3x10^{-1}$ per (mg/kg/day).

2.3 MINIMAL RISK LEVELS

The liver is the most sensitive target organ for carbon tetrachloride toxicity. Consequently, all derived minimal risk levels (MRLs) for carbon tetrachloride are based on nonneoplastic hepatic effects, which occurred at lower inhalation concentrations or oral doses compared to effects in other target tissues. Furthermore, all derived MRLs are based on rat studies since the observed nonneoplastic hepatic effects (fatty degeneration, necrosis, fibrosis, and cirrhosis) in this species are similar to the range of histopathology observed in exposed humans. Conversely, in exposed mice, the most significant nonneoplastic features of hepatic histopathology are fatty degeneration and necrosis, but not fibrosis or cirrhosis. Thus, studies in rats would appear to be preferred as a basis for human health risk assessment for carbon tetrachloride. The MRLs for carbon tetrachloride were the lowest available LOAELs for hepatic effects or the associated NOAELs (if available) in well-designed studies.

Inhalation MRLs

Inadequate human data indicated a NOAEL of 10 ppm and a LOAEL of 50 ppm for hepatic effects (decreased serum iron levels) following single exposures of six volunteers to carbon tetrachloride vapor lasting 1–3 hours (Stewart et al. 1961); the significance of serum iron to hepatic toxicity is not clear. A NOAEL for hepatic effects was not observed in acute-duration inhalation studies in animals. Adams et al. (1952) exposed male or female Wistar rats (2–30 of one sex/group) to carbon tetrachloride vapor at concentrations of 0, 10, 25, 50, 100, 200, or 400 ppm, 7 hours/day, 5 days/week for 5–15 exposures. A LOAEL of 10 ppm (2 ppm duration adjusted), the lowest concentration tested, was identified for slight fatty degeneration of the liver in 18 male Wistar rats exposed 7 hours/day for 13 days in a 17-day period. The extent and severity of fatty degeneration increased at ≥25 ppm. Cirrhosis of the liver occurred at ≥200 ppm and parenchymatous degeneration of renal tubules occurred in female rats treated at 400 ppm. Mild liver effects (altered glycogen distribution, hepatocytic steatosis, hydropic degeneration, and

necrosis, and elevated serum alanine aminotransferase) were observed in rats exposed at 50 ppm (12.5 duration adjusted) for 6 hours/day for 4 days (David et al. 1981). In another acute rat study, 100 ppm, the lowest concentration administered 8 hours/day, 5 days/week for 2 weeks, was also a LOAEL for hepatic effects (fatty degeneration and elevated serum sorbitol dehydrogenase) (Paustenbach et al. 1986a). Exposure for 15 minutes at 180 ppm resulted in a LOAEL for hepatic effects (increased alanine aminotransferase and relative liver weight) in rats (Sakata et al. 1987).

No MRL was established for acute-duration inhalation exposure to carbon tetrachloride because a derivation based on the most suitable data (the minimal LOAEL of 10 ppm in rats reported by Adams et al. 1952) would result in an acute-duration MRL lower than the intermediate- and chronic-duration MRLs. The intermediate-duration inhalation MRL of 0.03 ppm, based on a NOAEL of 5 ppm (Adams et al. 1952) is expected to be protective for acute-duration inhalation exposure.

- An MRL of 0.03 ppm has been derived for intermediate-duration inhalation exposure to carbon tetrachloride.

This MRL was calculated using a NOAEL of 5 ppm (1 ppm, adjusted for intermittent exposure), based on the absence of liver effects in Wistar rats (Adams et al. 1952). Wistar rats (15/sex/group) were exposed to carbon tetrachloride vapor at concentrations of 0, 5, 10, 25, 50, 100, 200, or 400 ppm, 7 hours/day for periods between 173 and 205 days. Fatty degeneration and increased liver weights were evident at concentrations of ≥10 ppm and cirrhosis occurred at ≥50 ppm. Hepatic effects have been reported with similar NOAELs and LOAELs for the guinea pig and Long-Evans or Sprague-Dawley rats (Adams et al. 1952; Prendergast et al. 1967). In monkeys, the NOAELs and LOAELs for hepatic effects were higher, 50 and 100 ppm, respectively (Adams et al. 1952; Smyth et al. 1936). Another intermediate-duration study in rats exposed 6 hours/day, 5 days/week reported granulation as the most sensitive effect at 10 ppm (the lowest concentration tested), fatty change at 30 ppm, and fibrosis and cirrhosis at 270 ppm (Japan Bioassay Research Center 1998). Mice exposed in the same study (Japan Bioassay Research Center 1998) showed a different array of hepatic effects: unspecified cytological alterations at 10 ppm and hepatic collapse at 30 ppm. The rat study by Adams et al. (1952) was selected as the basis for the intermediate-duration inhalation MRL because it offered the longest exposure duration and provided a NOAEL for hepatic toxicity based on the most sensitive LOAEL. A total uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) was applied to the duration-adjusted NOAEL (1 ppm for rats) to derive the MRL. An uncertainty factor of 3 for extrapolation from animals to humans was selected because rats are more sensitive to carbon tetrachloride toxicity than humans. Based on comparative PBPK modeling, Thrall et al. (2000) calculated that the metabolism of carbon

tetrachloride—which is the basis for its toxicity—proceeds at a higher rate in rats compared to humans (see Section 3.4.3).

- An MRL of 0.03 ppm has been derived for chronic-duration inhalation exposure to carbon tetrachloride.

This MRL was calculated using a NOAEL of 5 ppm (0.9 ppm, adjusted for intermittent exposure) and a LOAEL of 25 ppm based on hepatic effects in rats in a 2-year study (Japan Bioassay Research Center 1998; Nagano et al. 1998). Groups of Fischer 344 rats (50/sex/group) were exposed to carbon tetrachloride vapor at concentrations of 0, 5, 25, or 125 ppm for 6 hours/day, 5 days/week for 104 weeks. At 25 ppm, liver histopathology (fibrosis, cirrhosis, fatty change, granulation, foci, and deposition of ceroid) and statistically significant elevations in serum parameters (total bilirubin, serum glutamic-oxaloacetic transaminase [SGOT], and alanine aminotransferase) were observed in male and female rats. In the parallel assay in BDF1 mice, there is some uncertainty as to the apparent NOAEL of 5 ppm because the control values for serum chemistry parameters in males were unusually high compared to the companion subchronic study (no historical control values were available). At 25 ppm, hepatic degeneration and thrombus were evident in both sexes, and hepatic necrosis was found in female mice treated at ≥25 ppm. The rat study conducted by the Japan Bioassay Research Center (1998) was selected as the basis for the chronic-duration inhalation MRL because it provided a distinct NOAEL for hepatic effects. For calculating the MRL, a total uncertainty factor of 30 was applied to the duration-adjusted NOAEL of 0.9 ppm (3 for extrapolation between animals to humans and 10 for human variability). An uncertainty factor of 3 for extrapolation from animals to humans was selected because rats are more sensitive to carbon tetrachloride toxicity than humans. Based on comparative PBPK modeling, Thrall et al. (2000) calculated that the metabolism of carbon tetrachloride—which is the basis for its toxicity—proceeds at a higher rate in rats compared to humans (see Section 3.4.3).

Oral MRLs

- An MRL of 0.05 mg/kg/day has been derived for acute-duration oral exposure to carbon tetrachloride.

This MRL was calculated using a minimal LOAEL of 5 mg/kg/day, based on hepatic effects (Smialowicz et al. 1991). Fischer rats (three males/group) were orally dosed with 0, 5, 10, 20, or 40 mg/kg/day for 10 consecutive days; another set of animals (three males/group) was exposed with the addition of a 160 mg/kg/day group and evaluated for immunotoxicity. Several end points indicated progressive, dose-related liver injury. Centrilobular vacuolar degeneration was barely detectable in all six animals of the

5 mg/kg/day group (none was observed in any of the six controls), but became more severe as the dose was increased. Hepatocellular necrosis became evident first at 10 mg/kg/day, also becoming more pronounced with increasing dose. At higher doses, serum levels of alanine and aspartate aminotransferase became significantly elevated ($p<0.01–0.05$) (20 and 40 mg/kg/day), as did relative liver weight (40 mg/kg/day). No renal effects were observed at the highest dose of 40 mg/kg/day and no immunological effects were observed at doses as high as 160 mg/kg/day. Hepatic effects (cytoplasmic vacuolization and increased serum enzymes) have been reported in other studies at doses as low as 10 or 20 mg/kg/day, where those were the lowest doses tested (Bruckner et al. 1986; Kim et al. 1990b; Korsrud et al. 1972). The study of Smialowicz et al. (1991) was selected as the basis for the acute-duration oral MRL because it provided the lowest LOAEL for hepatic effects. For calculating the MRL, a total uncertainty factor of 100 was applied to the LOAEL of 5 mg/kg/day (3 for the use of a minimal LOAEL, 3 for extrapolation between animals to humans, and 10 for human variability). An uncertainty factor of 3 for extrapolation from animals to humans was selected because rats are more sensitive to carbon tetrachloride toxicity than humans. Based on comparative PBPK modeling, Thrall et al. (2000) calculated that the metabolism of carbon tetrachloride—which is the basis for its toxicity—proceeds at a higher rate in rats compared to humans (see Section 3.4.3).

- An MRL of 0.02 mg/kg/day has been derived for intermediate-duration oral exposure to carbon tetrachloride.

This MRL was calculated using a NOAEL of 1 mg/kg/day (0.71 mg/kg/day, adjusted for intermittent exposure), based on the absence of adverse hepatic effects detected at 10 mg/kg/day (Bruckner et al. 1986). Male Sprague-Dawley rats were exposed to 0, 1, 10, or 33 mg/kg/day, 5 days/week for 12 weeks, by corn oil gavage. Slightly elevated blood levels of sorbitol dehydrogenase and mild centrilobular vacuolation of the liver were observed at a LOAEL of 10 mg/kg/day, but not at 1 mg/kg/day. Cirrhosis, extensive degenerative hepatic lesions, and significantly elevated serum enzyme levels (ornithine carbamyl transferase and alanine aminotransferase) were observed at the high dose of 33 mg/kg/day. No renal effects were observed at the highest dose of 33 mg/kg/day. In mice exposed by gavage 5 days/week for 13 weeks, the NOAEL was 1.2 mg/kg/day and the LOAEL was 12 mg/kg/day for elevated serum enzymes and mild hepatic necrosis (Condie et al. 1986; Hayes et al. 1986). The study of Bruckner et al. (1986) was selected as the basis for the intermediate-duration oral MRL because it provided the most suitable NOAEL value for hepatic effects. For calculating the MRL, a total uncertainty factor of 30 was applied to the duration-adjusted NOAEL of 0.1 mg/kg/day (3 for extrapolation between animals to humans and 10 for human variability). An uncertainty factor of 3 for extrapolation from animals to humans was selected because rats are more sensitive to carbon tetrachloride toxicity than humans. Based

on comparative PBPK modeling, Thrall et al. (2000) calculated that the metabolism of carbon tetrachloride—which is the basis for its toxicity—proceeds at a higher rate in rats compared to humans (see Section 3.4.3).

No data were located on effects of chronic-duration oral exposure in humans or animals. A chronic MRL for this exposure route has not been derived.